Published in final edited form as:

Curr Protoc Immunol. 2010 August ; CHAPTER: Unit-7.37. doi:10.1002/0471142735.im0737s90.

Generation, Isolation, and Maintenance of Human Mast Cells and Mast Cell Lines

Madeleine Rådinger^{*}, Bettina M. Jensen^{**}, Hye Sun Kuehn^{*}, Arnold Kirshenbaum^{*}, and Alasdair M. Gilfillan^{*}

^{*}Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive MSC 1881, Bethesda, MD 20892-1881, USA.

^{**}Allergy Clinic - 7551, Copenhagen University Hospital, Tagensvej 20, DK-2200 Copenhagen N, Denmark.

Abstract

Antigen-mediated mast cell activation is a pivotal step in the initiation of allergic disorders including anaphylaxis and atopy. To date, studies aimed at investigating the mechanisms regulating these responses, and studies designed to identifying potential ways to prevent them, have primarily been conducted in rodent mast cells. However, to understand how these responses pertain to human disease, and to investigate and develop novel therapies for the treatment of human mast cell-driven disease, human mast cell models may have greater relevance. Recently, a number of systems have been developed which allow investigators to readily obtain sufficient quantities of human mast cells to conduct these studies. These mast cells release the appropriate suite of inflammatory mediators in response to known mast cell activators including antigen. These systems have also been employed to examine the signaling events regulating these responses. Proof of principle studies have also demonstrated utility of these systems for the identification of potential inhibitors of mast cell activation and growth. In this unit, we describe techniques for the development and culture of human mast cells from their progenitors and the culture of human mast cell lines. The relative merits and drawbacks of each model are also described.

Key Terms

FcεRI; Kit; Mast Cells; Human mast cells; CD34⁺ peripheral blood progenitor cells; HMC1 cells; LAD2 cells

This unit presents protocols for the culture of mast cells from human peripheral blood and cord blood as well as for available human mast cell lines. For protocols for the isolation and culturing of mast cells from rodents, readers are referred to Unit 3.23. The cells obtained from the protocols described in this unit can be used for investigating mast cell growth and survival, receptor-induced mediator release, receptor-dependent chemotactic responses, and the signaling pathways regulating these processes. Techniques for measuring mediator release from these cells are detailed in the “Measuring Mast Cell Mediator Release” unit. The relative merits and drawbacks for each cell type are also discussed.

The protocols described in this unit will allow the reader to obtain relatively pure populations of human mast cells from CD34⁺ peripheral blood progenitor cells, and from CD133⁺ cord blood progenitor cells. Techniques for culturing the LAD2 and HMC1 human mast cell lines are also described. Details are also provided about the characterization of the isolated and cultured cells by cellular staining with toluidine blue and cell surface molecules by flow cytometry.

NOTE: Unless indicated, all tissue culture supplies and pipettes are sterile disposable plasticware obtained from the following suppliers: Falcon (tubes and tissue culture dishes and plates), Corning (pipettes, tissue culture dishes, and plates), Nalgene (filters), Sarstedt (tissue culture flasks) and Nunc (tissue culture flasks).

Basic Protocol 1

Growth and culture of primary human mast cells from peripheral blood-derived CD34⁺ cells

Mast cells are derived from CD34⁺ hematopoietic progenitor cells that are defined as KIT (CD117)⁺ and CD13⁺, but FcεRI⁻ cells (Kirshenbaum et al. 1999). Undifferentiated mast cell progenitors leave the bone marrow and undergo their terminal differentiation in tissues; consequently, very few mature mast cells are found circulating in the peripheral blood. Even in their resident tissues, mast cells are only found in limited numbers, thus, primary human mast cells are difficult to isolate. An alternative means of obtaining reasonable populations of primary human mast cells has been by *in vitro* differentiation of CD34⁺ progenitors. CD34⁺ peripheral blood-derived human mast cells have the appearance of mature human mast cells having a well condensed non-lobate nucleus and abundant granules in their cytosol. They express FcεRI, KIT, and various G protein-coupled receptors (GPCRs), and respond, through these receptors to promote degranulation and cytokine production or, in the case of KIT or specific GPCRs in conjunction with FcεRI, to synergistically enhance these responses (Gilfillan et al. 2006, Kuehn et al. 2007). A number of toll-like receptors (TLRs) are also expressed on mast cells and, when activated, have the capacity to enhance antigen-mediated cytokine production (Qiao et al. 2006).

An increasing volume of literature has described the signaling events associated with these responses. These studies have revealed that the mechanisms regulating human mast cell activation are largely similar to those reported in the rodent systems (Gilfillan et al. 2006, Rivera et al. 2006). Our unpublished observations (Kuehn and Gilfillan), however, suggest that certain GPCR-mediated responses observed in the mouse may not necessarily be predictive of those observed in human mast cells. This supports the merit of utilizing human systems for examining the role of particular receptors and signaling in the context of human disease. We have indeed utilized CD34⁺ peripheral blood - derived human mast cells for examining potential inhibitors of KIT and FcεRI mediated mast cell activation (Jensen et al. 2008). Recently technologies have also been developed for gene knock down approaches for determining the relevance of specific signaling molecules in mast cell function in these cells (Furumoto et al. 2006). The major drawback for the CD34⁺ positive mast cells is the expense of culture and the obtaining of CD34⁺ progenitor cells.

Materials

Human mast cell culture medium: 500 ml StemPro-34 (GIBCO), 13 ml StemPro-34 Nutrient Supplement (GIBCO), l-Glutamine (2 mM) (GIBCO), Penicillin (100 U/ml)/Streptomycin (100 µg/ml) (GIBCO), Recombinant human Stem cell factor (rhSCF) (100 ng/ml),

Recombinant human Interleukin 6 (rhIL-6) (100 ng/ml), Recombinant human Interleukin 3 (rhIL-3) (30 ng/ml) (PeproTech)

All components are combined and then sterile filtered using a 0.2 µm filter.

Note: rhSCF, rhIL-6 and rh-IL-3 are aliquoted as 1000× solutions in distilled water which are stored at –80 °C.

Note: Human mast cell culture medium can be stored for 2–3 weeks at 4 °C. Cytokines are generally added freshly prior to use. Media containing cytokines can be stored for up to 2 weeks at 4 °C prior to use.

*NOTE: Human mast cell media containing 30 ng/ml IL-3 (in addition to 100 ng/ml SCF and 100 ng/ml IL-6) is only used for start up culture (i.e. first week of culture). Therefore, it is enough to make 100 ml of human mast cell media containing IL-3, IL-6 and SCF. When cells are fed **after** the initiation of cell culture (i.e. week 1–8), IL-3 is **NOT** added to the medium.*

15 ml conical polypropylene centrifuge tubes (e.g., Falcon)

50 ml conical polypropylene centrifuge tubes (e.g., Falcon)

Frozen aliquot of cells (1×10^7 CD34⁺ cells)

Tabletop centrifuge

Culture flasks T175 (e.g., Nunc)

5 ml polystyrene round-bottom tubes

PBS + 0.1% BSA (4 °C)

Anti-FcεRI- APC (eBioscience)

Mouse IgG-APC (eBioscience)

Anti-CD117-PE (BD Pharmingen)

Mouse IgG₁-PE (BD Pharmingen)

Mota's fixative

Add 4 g of lead acetate to 50 ml of deionized water. Mix by stirring at slow speed and add 2–4 ml of glacial acetic acid to dissolve the lead acetate. Finally, add 50 ml of absolute ethanol. Keep tightly closed and store at RT for 1–2 month.

Toluidine blue solution

Add 0.5 g of toluidine blue to 30 ml of absolute ethanol. Bring the volume up to 100 ml by adding deionized water. Adjust pH < 1 with 1N HCl. This solution can be stored at RT.

Distilled water

Ethanol (100%)

HCl

Cytospin slides

Cytocentrifuge (cytospin/Shandon)

Coverslip

Permout

Procedures—

1. Thaw an aliquot of CD34⁺ cells at 37° C. As soon as defrosted, transfer cells to a 15 ml tube. Rinse the cryotube with human mast cell medium and add to the 15 ml tube.
2. Centrifuge the cells at (450 × g) 5 min at room temperature (RT). Pipette off the medium and resuspend the cell pellet in 10 ml human mast cell media and transfer to two (i.e. 5ml to each flask) T175 culture flasks with enough medium to produce a final volume of 30–40 ml.
3. Check cells daily in the beginning of culture. Do not allow the cell density to exceed 0.5×10⁶ cells/ml. Usually, after 4–7 days in culture, the 2 flasks need to be divided into 6 flasks by adding 60 ml human mast cell medium (without IL-3) to each of the two flasks and transferring 30 ml of the resulting cell suspension into each of the additional 4 flasks.
4. After 14 days of culture, 30 ml of fresh human mast cell culture media (without IL3) is added to each of the 6 flasks then 30 ml of the resulting cell suspension removed and put into a further 6 new flasks resulting in a total of 12 flasks. However, this may vary within different batches of CD34⁺ cells.
5. At the third week of culturing, transfer cells from each flask to 50 ml falcon tube and spin down at 450 × g, 5 min at RT. Remove half of the supernatant, resuspend the cell pellet and transfer to a new T175 cell culture flask containing the equivalent volume of fresh human mast cell medium containing rhSCF and rhIL-6 (100 ng/ml, respectively). This is repeated weekly.

Note: Avoid repeated pipetting up and down to resuspend cells as the cells are easily damaged

6. After 7–8 weeks, the culture consists of mature human mast cells. The purity is evaluated by toluidine blue staining and / or flow cytometric analysis. At this point, the cells can be utilized for experiments and are used until week 10.

NOTE: It is important to change the flasks when changing media at week 3 and 4 of culture to remove any attached cells.

NOTE: Between weeks 0–2 there is a marked cell proliferation but around 3 weeks of culturing, cells begin to die off. At this point there may appear to be a lot of cell debris but this will eventually disappear during the subsequent weeks. Do not centrifuge the cell cultures before 3 weeks of age.

Determination of mast cell purity—Mast cell purity can be determined by 1) surface staining of FcεRI and KIT using flow cytometric analysis or; by 2) toluidine blue staining.

Surface staining of FcεRI and KIT: Mast cell purity can be determined by surface staining of FcεRI and KIT using flow cytometric analysis as follows:

1. At least 1×10^5 cells should be used per sample. Centrifuge for 5 min at $450 \times g$ at room temperature.
2. Resuspend cells with PBS + 0.1% BSA (4°C) to a density of 1×10^6 cells/ml, then add 100 μ l of the cell suspension per flow tube (5 ml polystyrene round-bottom tube).
3. Add anti-Fc ϵ RI-APC (or its isotype control; mouse IgG-APC) or anti-CD117-PE (or its isotype control; mouse IgG₁-PE) and incubate for 1 h at 4 °C in the dark.
Note: The amount of antibody is approximately 1 μ l of anti-CD117-PE and 5 μ l of anti-Fc ϵ RI-APC, but antibody titration is recommended before the start of the experiment.
4. Wash off excess antibodies by adding 1 ml PBS + 0.1% BSA to each tube and centrifuge for 5 min at $450 \times g$ at 4 °C. Resuspend cell pellet in 100 μ l of the PBS + 0.1% bovine serum albumin (BSA) buffer and analyze the cells on a flow cytometer.

Toluidine blue stain:

1. Spin down cells ($2\text{--}5 \times 10^4$ cells/cytospin slide) at $450 \times g$, 5 min at RT. Resuspend cells in 100 μ l PBS (optionally containing 25 μ l 10% BSA) per slide.
2. Add 100 μ l cell suspension to a cytopsin sample chamber with a clean slide. Spin slides at 550 rpm for 5 min in a cytocentrifuge (Shandon).
3. Allow the slides to air dry then fix the cells in Mota's fixative for 15 min. This can be done by adding dropwise to slides or alternatively in 50 ml tubes by submerging slides in the fixative.
4. Rinse cytopsin by gently dipping several times in distilled water replacing the water after each rinse. Air dry for at least 15 min and immerse in toluidine blue solution for 60 min.
5. Rinse off the excess stain by gently dipping in distilled water. Repeat with fresh water until no blue color is visible in the rinse and let slides air dry before mounting cover slip using Permount.

Basic Protocol 2

Generation and culture of cord blood-derived human mast cells

Cord blood contains a higher concentration of progenitors than does peripheral blood, which makes it a convenient progenitor source for mast cell generation. Both mononuclear cells and purified CD34⁺ and CD133⁺ progenitors have been used, however, this protocol will only describe the use of CD133⁺ cells due to the authors experience.

The mast cell yield is generally much higher when using cord blood progenitors compared to peripheral blood progenitors. An estimate is 100 cord blood derived mast cells / progenitor cell compared to 3.2 peripheral blood mast cells / progenitor cell, when working with non-mobilized peripheral blood progenitors (Andersen et al. 2008). However, the mast cells generated from cord blood appear more immature when compared to peripheral blood derived mast cells (see Background).

The major drawbacks for the CD133⁺-derived cord blood mast cells besides immaturity are similar to that for the CD34⁺-derived peripheral blood mast cells, namely, the access to cord blood can be a problem and the expense with culturing is high.

Materials

Cord blood progenitor preparation: 100 ml heparinized cord blood obtained within 30 min of newborn delivery

PBS

Ficoll-Paque (Amersham Pharmacia Biotech)

CD133 MicroBead kit (Miltenyi Biotech)

LS⁺ Separation column (Miltenyi Biotech)

MACS buffer (PBS containing 0.5% BSA, 2mM EDTA) – keep at 4 °C

MACS Separator for LS columns

15 ml conical polypropylene centrifuge tubes (e.g., Falcon)

Human cord blood mast cell culture medium: 500 ml StemSpan Serum-Free Expansion Medium (StemCell Technologies), Penicillin (100 U/ml)/ Streptomycin (100 µg/ml) (GIBCO), rhSCF (100 ng/ml) (R&D Systems, alternatively PeproTech), rhIL-6 (50 ng/ml) (R&D Systems, alternatively PeproTech), rhIL-3 (1 ng/ml) (R&D Systems, alternatively Peprotech) – only present first 3 weeks.

Fetal calf serum (GIBCO) - added to the culture medium from week 6 Medium stored at 4 °C, pre-warm to 37 °C before use. The medium can be store for 2 weeks at 4 °C.

Note: rhSCF, rhIL-6 and rh-IL-3 can be aliquoted as 1000× solutions and stored at –80 °C.

Additional materials: 15 ml and 50ml conical polypropylene centrifuge tubes (e.g., Falcon)

Tabletop centrifuge

Culture flasks T175 (e.g., Nunc)

Procedure—

1. Dilute heparinized cord blood in three volumes of PBS, overlay on a Ficoll-Paque gradient, then centrifuge at room temperature for 30 min at 450 × *g*.
2. Harvest the interface layer (mononuclear cells) and wash the cells three times in PBS at 4 °C, 5 min, 450 × *g*.
3. The CD133 cells are now purified using the CD133 MicroBead kit:
 - Resuspend cells in 300 µl cold MACS buffer per 1×10⁸ cells and add 100 µl FcR Blocking Reagent and 100 µl CD133 MicroBeads per 1×10⁸ cells. Mix well and incubate for 30 min at 4 °C.
 - Wash cells by adding 1–2 ml cold MACS buffer per 1 × 10⁸ cells (300 × *g*, 10 min) and resuspend cells in 500 µl per 1×10⁸ cells.
 - Place the LS column in the magnetic field of a MACS Separator. Prepare the column by rinsing with 3 ml of cold MACS buffer.
 - Apply cell suspension onto the column. Collect flow-through which is unlabeled cells (not the CD133⁺).

- Apply 3 ml cold MACS buffer onto the column and let it run through. Repeat this 3 times.
 - Remove column from magnetic field (separator) and place it on a 15 ml conical polypropylene centrifuge tube. Pipette 5 ml cold MACS buffer onto column and flush out the CD133⁺ cells by firmly pushing the plunger into the column.
4. Cell viability can be determined using trypan blue stain (0.5% in saline).
 5. The purified CD133⁺ cells are kept at a cell density 5×10^5 cell/ml in human cord blood mast cell culture medium (StemSpan culture medium supplemented with 100 ng/ml rhSCF, 50 ng/ml rhIL-6, 1 ng/ml rhIL-3 and Penicillin (100 U/ml) + Streptomycin (100 µg/ml)). After 3 weeks, rhIL-3 is omitted. From week 6, fetal calf serum (10 %) is added and mature mast cells are obtained after week 7.
 6. Medium is completely renewed weekly. Cells are spun down at 4 °C, 5 min, $450 \times g$, the supernatant gently aspirated and replaced with fresh medium to a cell density of 5×10^5 cell/ml.
 7. The mast cells can be utilized for experiments until the age of 10 weeks.

Mast cell purity can be determined by surface staining of FcεRI and KIT, and toluidine blue stain as described in Basic Protocol 1.

Basic Protocol 3

CULTURING OF LAD2 cells

The cell line that probably has the greatest utility for studies on human mast cell biology is the LAD 2 cell line, developed in the Laboratory of Allergic Diseases at NIAID/NIH from a patient with mastocytosis (Kirshenbaum et al. 2003). However, unlike the HMC-1 cell line, the LAD2 cells do not have an activating mutation in KIT, thus the LAD2 cells are an SCF-dependent cell line. LAD2 cells express FcεRI and contain abundant granules and, thus, degranulate well in response to antigen. Hence these cells can be used for the examination of signaling events regulating these processes. Furthermore, they may be suitable for screening potential inhibitors of mast cell degranulation. It has been our personal experience however that cytokine generation is deficient in these cells, especially when compared to that observed in primary cultures of human mast cells derived from CD34⁺ progenitor cells as described below. The other major drawback of the LAD2 cells however is the relatively slow growth rate with a doubling rate of approximately 2 weeks.

Materials

LAD2 Cell culture medium: 500 ml StemPro-34 (GIBCO), 13 ml StemPro-34 Nutrient Supplement (GIBCO), l-Glutamine (2 mM) (GIBCO), Penicillin (100 U/ml)/Streptomycin (100 µg/ml) (GIBCO), rhSCF (Peprotech) (100 ng/ml)

15 ml conical polypropylene centrifuge tubes (e.g., Falcon)

Frozen aliquot of cells

Tabletop centrifuge

Shaker platform

Sterile 6 well plate

Culture flasks

Equipment for counting cells using a hemacytometer

NOTE: All media components are added and then sterile filtered using a 0.2 µm filter and store at 4 °C. The medium is pre-warmed to 37 °C before use.

NOTE: LAD2 cells undergo osmotic shock and lyse easily, so directions should be followed accordingly. Slow equilibration in the freezing media (pZerve) prior to 37 °C should prevent lysis.

Procedure—

1. Immediately after thawing cells, check viability. Proceed if greater than 80–90%.
2. Leave cells in pZerve and add 100–200 µl StemPro-34 containing 200 ng/ml SCF and oscillate cells at 60 rpm in a 6 well plate on a shaker platform for 6 h at RT. However, check every 30 min for clumps or debris. Break up clumps by gently pipetting, or remove clumps and debris.
3. After 6 h, if cell viability is adequate, add additional 500 µl StemPro-34 containing 200 ng/ml SCF and transfer to 37 °C.
4. Change media within 24 h by spinning cells at $200 \times g$ for 5 minutes at room temperature. Cells initially grow slowly, but should start doubling within 2–4 weeks.
5. Replace half of the media weekly by adding equal volume of fresh StemPro-34 containing 100 ng/ml SCF (i.e. hemi-depletion). Careful pipetting of the media out of the flask along the flask wall is sufficient and an option to spinning cells and decanting supernatant.
6. Cryopreserve cells: Whenever cell numbers permit, LAD2 cells should be aliquoted and cryopreserved. It is also best to thaw and start new stocks of LAD2 cells every 12 months. For cryopreservation, prepare a cell pellet of 1×10^7 cells per cryotube by centrifugation. Leave a small amount (50–100 µl) of StemPro-34 containing SCF (100–200 ng/ml). Resuspend cells in pZerve cryopreservative supplemented with 200 ng/ml SCF in a volume of 1.5 ml. Suspend cells for 30 min at RT with gently shaking, place in Nalgene cryo container, transfer to –20 °C for 1 hour, then –80 °C for 1 h and then place in liquid nitrogen.

NOTE: LAD2 cells should be well mixed by gently pipetting prior to freezing.

Basic Protocol 4

Culturing of the HMC1.1 and HMC1.2 cell lines

The HMC-1 cell line is a growth factor-independent human mast cell line developed in the laboratory of Dr. Joseph H Butterfield (the Mayo Clinic). These cells, were derived from a patient with mast cell leukemia (Butterfield et al. 1988). Two subtypes of the HMC-1 cells were subsequently delineated on the basis of the presence of the specific activating mutations present in the cytosolic domain of KIT: HMC-1.1 which contains a mutation within the juxta-membrane region (V560G); and HMC-1.2 which contains both this mutation and the D816V mutation in the catalytic domain (Sundstrom et al. 2003) which has been associated with the mast cell myeloproliferative disease, mastocytosis (Metcalf 2008). Although these cells have been useful to explore certain mast cell activations events, for example the influence of adenosine and other GPCR agonists on cytokine production

(Ryzhov et al. 2006), they only poorly express the FcεRI and are also poorly granulated, (Butterfield et al. 1988) thus are not ideal for examining antigen-mediated degranulation.

Materials

HMC1.1 and 1.2 Cell culture medium: 435 ml Iscove's medium (Iscove's Modified Dulbecco's Medium: IMDM containing 25 mM HEPES), FBS (10%), L-Glutamine (2 mM) (GIBCO), Penicillin (100 U/ml)/Streptomycin (100 µg/ml) (GIBCO)

15 ml conical polypropylene centrifuge tubes (e.g., Falcon)

Frozen aliquot of cells

Tabletop centrifuge

Culture flasks

Equipment for counting cells using a hemacytometer

NOTE: All medium components are combined then sterile filtered using a 0.2 µm filter then stored at 4 °C. The medium is pre-warm to 37 °C before use.

NOTE: All equipments and solutions coming into contact with living cells must be sterile and sterile technique should be used accordingly.

Procedure—

1. Thaw the cells and immediately transfer the cells suspension to a 15 ml conical tube.
2. Add 10 ml medium and centrifuge at $450 \times g$ for 5 min, room temperature (RT). Remove supernatant and resuspend in 10 ml culture medium.
3. Count cells and adjust cell concentration to 2×10^5 cells/ml.
4. Cells should be passaged every 3–5 day. Do not allow the cell density to exceed 2×10^6 cells/ml.

NOTE: Cryopreserve cells: Cell lines should not be subcultured for longer than 3 months, therefore routinely defrost fresh isolates and prepare fresh freeze downs ($1-5 \times 10^6$ cells).

COMENTARY

Background

Mast cells are tissue-resident cells of hematopoietic origin which contribute to adaptive and innate immune responses (Mekori et al. 2000, Metcalfe et al. 1997). However, it is their central role in generating and secreting the inflammatory mediators responsible for anaphylaxis and atopy that has primarily driven the research in the field of mast cell biology. Mast cells develop from CD34⁺/CD13⁺/CD117⁺ (KIT) progenitor cells which originate in the bone marrow then migrate to, and mature in their tissues of residence in response to the KIT ligand, stem cell factor (SCF) (Kirshenbaum et al. 1999, Metcalfe et al. 1997). Two subtypes of human mast cells have been designated based primarily on the protease contents of their granules: MC_{TC}, whose granules contain both tryptase and chymase, and MC_T whose granules primarily contain tryptase. The human MC_{TC} is considered to be more analogous to the connective tissue mast cells described in rodents, whereas the human MC_T subtype is more analogous to the mucosal mast cells described in rodents (Metcalfe et al. 1997).

Mature tissue-resident mast cells are principally activated via the high affinity receptor for IgE (FcεRI) following binding of antigen to antigen-specific IgE occupying these receptors (Kraft et al. 2007), resulting in the release of inflammatory mediators as described in the “Measuring Mast Cell Mediator Release” unit. This response however can be markedly upregulated or downregulated by ligation of various other receptors expressed on mast cells. For example Kit, and various G protein coupled receptors (GPCRs) and Toll-like receptor (TLRs), when activated, enhance mast cell degranulation (Kit, GPCRs) and/or cytokine production (Kit, GPCRs, TLRs), whereas other cell surface receptors such as other GPCRs, FcγRIIb, and other ITIM-containing inhibitory receptors can down-regulate FcεRI-mediated mast cell activation degranulation and cytokine production (Gilfillan et al. 2006).

The majority of studies on mast cells, to date, have been conducted in cells of rodent origin, particularly the RBL 2H3 rat mast cell line and mast cells derived from the bone marrow of wild type, transgenic and gene knockout mice. The primary reasons being the ease of obtaining large numbers of cells, ease of triggering the cells with available reagents, ease of transduction, and the ability to generate or obtain genetically modified mice allowing delineation of required signaling events. Until recently, studies on human mast cells have been limited; largely being restricted to cells derived from human tissue such as lung and skin following protease digestion and purification through cell separation gradients. The difficulty in maintaining consistency in sources of the starting tissues and the digestion and purification procedures however have the potential to compromise the viability of the mast cells isolated in this manner and to introduce significant batch to batch variability.

Studies aimed at the identification of mast cell progenitors and their requirements for subsequent differentiation, expansion, and development into mature mast cells have led to the evolution of protocols for that has allowed relatively high numbers of pure populations of human mast cells to be attained. The major sources for the progenitors obtained for these protocols have been cord blood and CD34⁺ progenitors obtained from peripheral blood. Peripheral blood contains a relatively low percentage of CD34⁺ cells (0.01–0.1%), therefore, it is necessary to enrich this population prior to growing the cells. CD34⁺ cells are commercially available, however, in our laboratory, we obtain the cells from normal donors, following G-CSF injection which mobilizes bone marrow progenitors. The normal donors are subjected to apheresis, and the CD34⁺ cells are purified by means of positive selection (all through a protocol approved by the NIAID/NIH IRB committee). We routinely obtain around $2\text{--}3 \times 10^8$ CD34⁺ cells per normal donor which are subsequently stored under liquid nitrogen until use.

Cord blood contains a high number of progenitor cells which makes this progenitor source favorable compared to peripheral blood. This difference in mast cell yield between cord blood and peripheral blood is, however, not related to progenitor type since the mast cell yield from peripheral blood do not increase when starting out with CD133⁺ cells (Holm et al. 2008). The high yield of mast cells makes the cord blood protocol amenable for studies like microarray analysis which needs a high cell number but it has to be taken into account that the cord blood derived mast cells are different from the peripheral blood derived. They contain less histamine, express fewer FcεRI and CD203c receptors but more CD117, and when activated through FcεRI they release less histamine, prostaglandin D₂ and cytokines (Andersen et al. 2008, Iida et al. 2001). It is therefore important to consider the mast cells generated from either cord blood or peripheral blood as two distinct types of mast cells.

Troubleshooting

As discussed in Unit 3.23, fungal, yeast, mycoplasma and bacterial contamination can present a problem, particularly as the human mast cell cultures take around 8 weeks before they are mature. Fungal, yeast, and bacterial infections are readily discernable by eye and mycoplasma

contamination which is usually suspected when cells are growing slower than expected, can be detected by commercially available kits. Should contamination occur, immediately bleach and discard all contaminated flasks and scrub and clean all surfaces that have contacted these flasks with 70% ethanol.

Evidence of slower than expected growth rates or signs of cell death during the later stages of growth may also indicate problems with the growth media or batches of cytokines or SCF. It is to be expected to see a substantial amount of debris around week 3 of culture, however, due to die off of the non-mast cell committed lineages.

LAD2 cells grown for prolonged periods that display excessive clumping or slower growth may lose responsiveness to biotinylated IgE/SA crosslinking, and have reduced activation and degranulation. Clumping of cells can be minimized by maintaining cell concentrations between $0.25\text{--}0.5 \times 10^6$ cells/ml and performing hemidepletions every 3–4 days. A complete media change to spin off cell debris should be done as necessary by spinning cells at $200 \times g$ for 5 minutes and replacing with fresh StemPro34 with rhSCF. Gentle pipetting of suspended cells with 1000 μ l pipette tips will break up most cell clumps. In the event that cell degranulation or growth rates do not improve, thaw out an aliquot of frozen cells and restart LAD2 cell stocks.

Anticipated results

For CD34⁺-peripheral blood derived human mast cells, we start with 1×10^7 CD34⁺ cells which after 8 weeks in culture yield approximately $3\text{--}10 \times 10^7$ mature human mast cells with a high degree of purity (~99%).

One hundred ml of cord blood gives around 1×10^6 CD133⁺ cells which proliferate into approximately 1×10^8 mast cells.

The doubling time for LAD2 cells is approximately 10–14 days and HMC1 cells, 1–2 days.

Time consideration

Growth and culture of primary human mast cells from 1×10^7 blood-derived CD34⁺ cells takes approximate 30 min to 1 hour a week, depending on the rate of growth, which may vary between different batches of CD34⁺ cells. The cells are mature after 7–8 weeks (with a purity >95%) at which point they can be used.

Purification of cord blood CD133⁺ cells takes approximately 3 hours. Culturing of the cells is similar to the peripheral blood CD34⁺ cells described above.

Starting up a culture of LAD2 cells take around 6 hours. The cells grow slowly initially, but should start doubling in 2–4 weeks after thawing. Cells need to be fed once a week which takes around 15–30 min depending on how many cultures you have.

Starting up a culture of HMC1 cell lines takes around 30 min. Cells expand fast and usually need to be passaged / fed every 3–5 days, which takes approximately 15–30 min depending on numbers of cultures.

Acknowledgments

Research in the authors' laboratory (MR, HSK, AK, and AMG) has been supported by funding from the National Institute of Allergy and Infectious Diseases Intramural research program, National Institutes of Health.

Literature cited

- Andersen HB, Holm M, Hetland TE, Dahl C, Junker S, Schiotz PO, Hoffmann HJ. Comparison of short term in vitro cultured human mast cells from different progenitors - Peripheral blood-derived progenitors generate highly mature and functional mast cells. *J.Immunol.Methods*. 2008; 336:166–174. [PubMed: 18538784]
- Butterfield JH, Weiler D, Dewald G, Gleich GJ. Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk.Res*. 1988; 12:345–355. [PubMed: 3131594]
- Furumoto Y, Brooks S, Olivera A, Takagi Y, Miyagishi M, Taira K, Casellas R, Beaven MA, Gilfillan AM, Rivera J. Cutting Edge: Lentiviral short hairpin RNA silencing of PTEN in human mast cells reveals constitutive signals that promote cytokine secretion and cell survival. *J.Immunol*. 2006; 176:5167–5171. [PubMed: 16621980]
- Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat.Rev.Immunol*. 2006; 6:218–230. [PubMed: 16470226]
- Holm M, Andersen HB, Hetland TE, Dahl C, Hoffmann HJ, Junker S, Schiotz PO. Seven week culture of functional human mast cells from buffy coat preparations. *J.Immunol.Methods*. 2008; 336:213–221. [PubMed: 18547584]
- Iida M, Matsumoto K, Tomita H, Nakajima T, Akasawa A, Ohtani NY, Yoshida NL, Matsui K, Nakada A, Sugita Y, Shimizu Y, Wakahara S, Nakao T, Fujii Y, Ra C, Saito H. Selective down-regulation of high-affinity IgE receptor (FcεRI) alpha-chain messenger RNA among transcriptome in cord blood-derived versus adult peripheral blood-derived cultured human mast cells. *Blood*. 2001; 97:1016–1022. [PubMed: 11159531]
- Jensen BM, Beaven MA, Iwaki S, Metcalfe DD, Gilfillan AM. Concurrent inhibition of kit- and FcεRI-mediated signaling: coordinated suppression of mast cell activation. *J.Pharmacol.Exp.Ther*. 2008; 324:128–138. [PubMed: 17925481]
- Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, Rao VK, Metcalfe DD. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcεRI or FcγRI. *Leuk.Res*. 2003; 27:677–682. [PubMed: 12801524]
- Kirshenbaum AS, Goff JP, Semere T, Foster B, Scott LM, Metcalfe DD. Demonstration that human mast cells arise from a progenitor cell population that is CD34(+), c-kit(+), and expresses aminopeptidase N (CD13). *Blood*. 1999; 94:2333–2342. [PubMed: 10498605]
- Kraft S, Kinet JP. New developments in FcεRI regulation, function and inhibition. *Nat.Rev.Immunol*. 2007; 7:365–378. [PubMed: 17438574]
- Kuehn HS, Gilfillan AM. G protein-coupled receptors and the modification of FcεRI-mediated mast cell activation. *Immunol.Lett*. 2007; 113:59–69. [PubMed: 17919738]
- Mekori YA, Metcalfe DD. Mast cells in innate immunity. *Immunol.Rev*. 2000; 173:131–140. [PubMed: 10719674]
- Metcalfe DD. Mast cells and mastocytosis. *Blood*. 2008; 112:946–956. [PubMed: 18684881]
- Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev*. 1997; 77:1033–1079. [PubMed: 9354811]
- Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA. FcεRI and toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. *Blood*. 2006; 107:610–618. [PubMed: 16174756]
- Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J.Allergy Clin.Immunol*. 2006; 117:1214–1225. [PubMed: 16750977]
- Ryzhov S, Goldstein AE, Biaggioni I, Feoktistov I. Cross-talk between G(s)- and G(q)-coupled pathways in regulation of interleukin-4 by A(2B) adenosine receptors in human mast cells. *Mol.Pharmacol*. 2006; 70:727–735. [PubMed: 16707627]
- Sundstrom M, Vliagoftis H, Karlberg P, Butterfield JH, Nilsson K, Metcalfe DD, Nilsson G. Functional and phenotypic studies of two variants of a human mast cell line with a distinct set of mutations in the c-kit proto-oncogene. *Immunology*. 2003; 108:89–97. [PubMed: 12519307]